Minimally Processed Vegetable Salads: Microbial Quality Evaluation

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ABSTRACT

The increasing demand for fresh fruits and vegetables and for convenience foods is causing an expansion of the market share for minimally processed vegetables. Among the more common pathogenic microorganisms that can be transmitted to humans by these products are *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella*. The aim of this study was to evaluate the microbial quality of a selection of minimally processed vegetables. A total of 181 samples of minimally processed leafy salads were collected from retailers in the city of Sao Paulo, Brazil. Counts of total coliforms, fecal coliforms, *Enterobacteriaceae*, psychrotrophic microorganisms, and *Salmonella* were conducted for 133 samples. *L. monocytogenes* was assessed in 181 samples using the BAX System and by plating the enrichment broth onto Palcam and Oxford agars. Suspected *Listeria* colonies were submitted to classical biochemical tests. Populations of psychrotrophic microorganisms $>10^6$ CFU/g were found in 51% of the 133 samples, and *Enterobacteriaceae* populations between $10^5$ and $10^6$ CFU/g were found in 42% of the samples. Fecal coliform concentrations higher than $10^2$ CFU/g (Brazilian standard) were found in 97 (73%) of the samples, and *Salmonella* was detected in 4 (3%) of the samples. Two of the *Salmonella*-positive samples had $<10^2$ CFU/g concentrations of fecal coliforms. *L. monocytogenes* was detected in only 1 (0.6%) of the 181 samples examined. This positive sample was simultaneously detected by both methods. The other *Listeria* species identified by plating were *L. welshimeri* (one sample of curly lettuce) and *L. innocua* (2 samples of watercress). The results indicate that minimally processed vegetables had poor microbiological quality, and these products could be a vehicle for pathogens such as *Salmonella* and *L. monocytogenes*.

Consumer demand for healthy, freshlike, and easy-to-prepare products coupled with consumer lifestyles changes have led to the development of a variety of novel products (8, 29). These products are processed and ready-to-eat (RTE) and take minimal time to prepare. Minimally processed vegetables (MPVs) are an example of such products. MPVs are submitted to processing that includes selection, washing, peeling, cutting, sanitizing or heat treating, and packaging (2). However these operations do not assure the absence of microorganisms or long-term stability of the product; therefore, MPVs must be stored under refrigeration (2, 19).

The physical structure of the vegetable and its chemical composition can induce different types of contamination and/or deterioration. Irrigation water, soil, and organic fertilizers are some of the possible sources of contamination in the fields (5). Seasonality also influences the microflora present in the product (6). Contamination also can occur during harvest, transport, processing, or storage.

Microorganisms that are adhered to the surface of freshly harvested vegetables are mainly gram-negative saprophytes. However, some human pathogens such as *Yersinia enterocolitica*, *Salmonella*, and enteropathogenic *Escherichia coli* also can be found (2, 6). Leafy vegetables that are in contact with the soil may harbor gram-positive bacteria such as *Bacillus cereus*, *Clostridium* spp., and *Listeria monocytogenes* (6). These microorganisms may survive washing and sanitizing steps (1) because they have formed biofilms on the surface of the vegetable or because they are protected by the serous cuticle of the vegetable (19).

Cut vegetables are more susceptible to chemical and microbiological deterioration because during cutting cells are destroyed and exudates rich in minerals, sugars, vitamins, and other compounds are released. These nutrients and conditions can allow growth of microorganisms that have survived processing (2, 19, 30).

Extension of the shelf life of MPVs by using passive or active modified atmosphere packaging has supported the usefulness of this processing method because it suppresses the growth of spoilage microorganisms. However, this technology may create conditions that are appropriate for slowly growing pathogenic bacteria (1) such as *L. monocytogenes*. Irradiation is another technology that can be used to increase the quality and safety of products (4, 13, 18, 20).

To determine the microbial quality of MPVs found in
TABLE 1. Populations of total and fecal coliforms, Enterobacteriaceae, and aerobic psychrotrophic bacteria in samples of minimally processed vegetables

<table>
<thead>
<tr>
<th>Population (CFU/g)</th>
<th>Coliforms</th>
<th>Fecal coliforms</th>
<th>Enterobacteriaceae</th>
<th>Aerobic psychrotrophic bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>3 (2.3)</td>
<td>36 (27.1)</td>
<td>1 (0.8)</td>
<td>3 (2.3)</td>
</tr>
<tr>
<td>10^2–10^3</td>
<td>0</td>
<td>1 (0.8)</td>
<td>0</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>10^3–10^4</td>
<td>15 (11.3)</td>
<td>38 (28.6)</td>
<td>15 (11.3)</td>
<td>7 (5.3)</td>
</tr>
<tr>
<td>10^4–10^5</td>
<td>29 (21.8)</td>
<td>35 (26.3)</td>
<td>34 (25.6)</td>
<td>15 (11.3)</td>
</tr>
<tr>
<td>10^5–10^6</td>
<td>57 (42.9)</td>
<td>3 (2.3)</td>
<td>56 (42.1)</td>
<td>39 (29.3)</td>
</tr>
<tr>
<td>&gt;10^6</td>
<td>28 (21.1)</td>
<td>1 (0.8)</td>
<td>22 (16.5)</td>
<td>68 (51.1)</td>
</tr>
</tbody>
</table>

TABLE 2. Incidence of fecal coliforms (populations >10^2 CFU/g), Salmonella, and L. monocytogenes in samples of ready-to-eat minimally processed vegetables

<table>
<thead>
<tr>
<th>Type of vegetable</th>
<th>Total no. of samples</th>
<th>Fecal coliforms (at &gt;10^2 CFU/g)</th>
<th>Salmonella (in 25 g)</th>
<th>L. monocytogenes (in 25 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettuce (differ-</td>
<td>41</td>
<td>25 (61)</td>
<td>1 (2.4)</td>
<td>0</td>
</tr>
<tr>
<td>enent types)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed salads</td>
<td>21</td>
<td>19 (90.5)</td>
<td>1 (4.8)</td>
<td>0</td>
</tr>
<tr>
<td>Watercress</td>
<td>13</td>
<td>11 (84.6)</td>
<td>1 (7.7)</td>
<td>0</td>
</tr>
<tr>
<td>Spinach</td>
<td>12</td>
<td>7 (58.3)</td>
<td>0</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Arugula</td>
<td>12</td>
<td>10 (83.3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chichories</td>
<td>12</td>
<td>9 (75.0)</td>
<td>1 (8.3)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>22</td>
<td>16 (72.7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>133</td>
<td>97 (73.0)</td>
<td>4 (3.0)</td>
<td>1 (0.8)</td>
</tr>
</tbody>
</table>

* Iceberg, Boston, and curly leafy lettuces.
* Composed mainly of lettuce plus others leaves.
* Broccoli, cabbage, kale, mustard leaves, and acelga.

RESULTS AND DISCUSSION

A total of 181 samples were analyzed for *L. monocytogenes* by using the BAX system or by plating the secondary enrichment. Of these samples, 133 had populations of psychrotrophic microorganisms, *Enterobacteriaceae*, nonfetal and fecal coliforms, and *Salmonella*.

*L. monocytogenes* was found in only 1 (0.6%) of the 181 samples, which was a sample of spinach. Other *Listeria* species were also detected by plating in a total of three samples (1.7%): *L. innocua* in two watercress samples and *L. welshimeri* in one sample of curly lettuce. The results for *L. monocytogenes* obtained by plating and by BAX System were in agreement. However, 82 colonies derived from the plating method from 14 other samples were submitted for identification, and none of them were identified as *L. monocytogenes*.

The occurrence of *L. monocytogenes* in this study is comparable to that found by others. In Canada, Farber et al. (11) did not detect *L. monocytogenes* in 110 samples of vegetables; however, they found *Listeria ivanovii* in one radish sample (0.9%). In the United States, Lin et al. (17) reported that 1.6% (1 of 63) of the salad samples analyzed contained *L. monocytogenes*. In Portugal, Guerra et al. (14) found *L. innocua* in 1 (4.3%) of 37 samples of fruits and vegetables but no *L. monocytogenes*.

Higher prevalence of *L. monocytogenes* in vegetables has been reported by other researchers. In Canada, Odu-meru et al. (22) evaluated 361 samples of vegetables (65 unprocessed and 296 RTE) and found *L. monocytogenes* in 18 (6.1%) of the RTE samples and 1 (1.5%) of the samples of unprocessed vegetables. In Brazil, Porto and Eiroa (25) analyzed 250 samples of different leafy vegetables and found *L. monocytogenes* in 3.2% of them. In the United Kingdom, Sagoo et al. (27) reported the presence of *Listeria* spp. and *L. monocytogenes* in 169 (4.4%) and 88 (2.3%), respectively, of the 3,852 RTE salads analyzed. In India, Pingulkar et al. (24) examined 128 samples of vegetables and found *L. monocytogenes* in 6 samples of tomatoes and 8 samples of 54 different vegetables; however, they did not detect this pathogen in RTE salads.

In the present study, aerobic psychrotrophic bacteria at >10^6 CFU/g were found in 68 (51.1%) of the samples, and *Enterobacteriaceae* at >10^3 CFU/g were present in 127 (95.5%) of the samples. In 56 samples (42.1%), the *Enterobacteriaceae* population was between 10^5 and 10^6 CFU/g (Table 1). Coliforms were present in 130 (97.7%) of the 133 samples, and fecal coliforms were identified in 97 (73%) of the 133 samples with populations >10^5 CFU/g (Table 1). Table 2 depicts the type of MPV and the number
of samples with populations of fecal coliforms >10^2 CFU/g.

Salmonella was detected in four samples (3%, Table 2): watercress, chicory, lettuce, and mixed salad (beets, carrots, lettuce, cabbage, and watercress). Two of the Salmonella-positive samples (chicory and lettuce) had populations of fecal coliforms <10^2 CFU/g.

Although the populations of Enterobacteriaceae and fecal coliforms found in the present study were high (>10^2 CFU/g), these groups of microorganisms are common in raw vegetables and are not necessarily associated with fecal contamination; the majority of the genera are part of the endogenous microflora of the product (6). According to these data, 99 samples (74.5%) were of unsatisfactory hygienic condition and did not meet the Brazilian Standard (7) for RTE MPVs, which is a maximum of 10^2 CFU/g for fecal coliforms and no Salmonella in 25 g of product.

Few published studies have dealt with the microbiological quality of natural and minimally processed vegetables. Results similar to the those found in this study were reported by Pingulkar et al. (24), who found fecal coliforms in 65% of the fresh vegetables evaluated. However, these authors did not report the population sizes. Garg et al. (12) found populations between 10^2 and 10^3 CFU/g of psychrotrophic microorganisms in lettuce, coleslaw, spinach, cauliflower, and carrot sticks. These authors concluded that high populations of psychrotrophic bacteria in MPVs can negatively affect the shelf life of these products. Sagoo et al. (28) detected Enterobacteriaceae in 2,919 (76%) of the 3,852 RTE salad samples, and the population was ≥10^4 CFU/g in 1,404 (37%) of these positive samples. Salmonella was isolated from five samples (0.13%). In another study Sagoo et al. (27) evaluated the quality of 2,950 pre-opened RTE MPV samples, Enterobacteriaceae was found in all samples, with populations ≥10^3 CFU/g in 1,638 (55.5%) of the samples. Salmonella was not detected in the examined samples.

The washing step during processing reduces the microbiological load in MPVs, but this reduction is generally limited and is inadequate as a single sanitation step. In studies by Goularte et al. (13) and Martins et al. (18), Enterobacteriaceae and coliform populations were reduced by approximately 1 log cycle during minimal processing of lettuce.

MPVs are expected to be consumed without any further treatment in the home. For this reason, care must be taken at the farm and during processing to assure maximum bacterial removal and to prevent cross-contamination and bacterial growth during production and storage. Good hygiene practices in the MPV processing continuum, from farm to table, must be followed. The quality of the raw material, the choice of products used for washing and/or sanitizing the MPVs, the type of packaging, and temperature control during storage and distribution also will affect the final quality. Our results indicate that RTE MPVs bought in São Paulo supermarkets are of poor microbiological quality and could be a vehicle for transmission of Salmonella or L. monocytogenes. However, L. monocytogenes does not seem to be a problem in these products. RTE MPV producers should pay more attention to the selection of raw materials and sanitizing steps to provide a safer product to the consumers. Processors also can use irradiation (in addition to good manufacturing practices) to control microorganisms in RTE MPVs (10, 18). This technology has proved safe and does not impair the sensory characteristics of these products when the correct dose is applied (13, 15, 18, 21, 26). If actions are not taken by producers, public health and food safety authorities must inform consumers about the potential risks associated with the consumption of RTE MPVs.

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